THE ASSOCIATION BETWEEN ACIDIFICATION AND ELECTROGENIC EVENTS IN THE RAT PROXIMAL JEJUNUM

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SUMMARY

1. Simultaneous measurement of hydrogen ion production, transmural potential difference and intermittent short-circuit current (SCC) was made in the rat proximal jejunum *in vitro*: similarly, potassium and sodium ion movements were measured to investigate the relationship of acidification to electrogenic events and associated ion movements in the jejunum.

2. Acidification correlated significantly with the short-circuit current and both were inhibited by 10 mM serosal ouabain or 10 mM mucosal aminophylline. Both inhibitors had effects on net potassium movement but not on net sodium movement. Moreover, in isotope studies whereas 10 mM serosal ouabain reduced the J_{ms} sodium flux, 10 mM mucosal aminophylline had no effect, i.e. aminophylline can reduce both short-circuit current and acidification without perceptibly altering the serosally directed sodium flux.

3. In low-sodium buffers in which acidification still occurs although reduced, transmural potential differences occur of reversed polarity that are apparently unrelated to sodium diffusion potential differences (as evidenced by isotopic sodium efflux experiments) and which could be caused by hydrogen ion production. In low sodium buffers however the inhibitors have opposing effects, ouabain causing an increase and aminophylline a decrease in the reversed potential differences.

4. A model for acidification (that of potassium rather than sodium ion exchange for the hydrogen ion and hydroxyl for chloride ion exchange) is proposed to explain the present experimental findings and other diverse observations in the literature: although either step might be the electrogenic step, acidification must be also considered as a component of the jejunal short-circuit current.

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INTRODUCTION

Recent experiments demonstrating a slight acid luminal pH in rat jejunum and a more alkaline pH in the ileum (Waldron-Edward, 1971) have confirmed original observations that showed regional differences in luminal pH (Redman, Willimot & Wokes, 1927; McRobert, 1928) apparent as a distally declining gradient of pH between lumen and plasma. The jejunal secretion of fluid of low pH *in vivo* was thought to be the underlying cause (Robinson, Luckey & Mills, 1943). Both *in vitro* and *in vivo*, rat jejunum can neutralize excessively acid or alkaline buffers instilled in the lumen (Ponz & Larralde, 1950; Foerster, Erdlenbruch & Mehnert, 1967). This implies, if not the existence of a homoeostatic mechanism designed to maintain luminal pH at characteristic regional values, then the gradual reassertion, by intestinal secretion, of the pH typical of that region of the intestine.

Despite the repeated observation of these luminal pH changes, mainly toward more acid values (M. E. Smith, personal communication; and Powell, Solberg, Plotkin, Catlin, Maenza & Formal, 1971; Hubel, 1973), literature dealing with acidification as such, is sparse. Luminal pH critically affects the transport of many compounds, including weak electrolytes whose transfer is passive: since this is so, acidification also should influence these various transport systems. Models have been proposed for facilitated weak electrolyte transfer, invoking an intermediate compartment of either alkaline pH (Jackson, Shiau, Bane & Fox, 1974) or particularly for folic acid (Benn, Swan, Cooke, Blair, Matty & Smith, 1971; Blair, Johnson & Matty, 1974) a model incorporating more recent details about acidification (Blair, Lucas & Matty, 1975) invoking a region of acid pH as proposed by Schanker, Tocco, Brodie & Hogben (1958). Although the concept of gastric acidification is a familiar one, intestinal acidification and its clinical aspects, together with its possible role in malabsorption syndromes, is almost completely unknown.

Acidification studies on amphibian membranes, including frog skin and turtle bladder, have suggested that both bicarbonate transfer (Brodsky & Schilb, 1972) and hydrogen ion secretion in the reverse direction (Steinmetz, 1967) might be the underlying cause. A sodium–hydrogen ion exchange presumed not to be electrogenic, has been proposed for proximal jejunum (Turnberg, Fordtran, Carter & Rector, 1970; Kloetz & Schloerb, 1971) in man and rat. The present work has investigated acidification in relation to electrical events (principally short-circuit current, SCC) and various ion movements in an attempt to both clarify the mechanism of acidification and test the postulated ion exchange models.

METHODS

Source of chemicals

Aminophylline was obtained from the Sigma Chemical Co., U.S.A., ouabain from Merck Ltd, as were all the inorganic reagents used in the buffer solutions. Sodium [^{14}C]bicarbonate was obtained from Hoechst Ltd, Frankfurt, ^{22}Na and ^{3}H from the Radiochemical Centre, Amersham, U.K.

Buffers

The principal buffer for acidification studies was a modified Krebs–Henseleit (Krebs & Henseleit, 1932) with all phosphate replaced by bicarbonate to maintain osmolarity, which when gassed with 95:5 (v/v) $O_2:CO_2$ mixture, gave a final pH around 7·2. Phosphate was excluded since jejunal transport of the anion would cause alterations in the buffering power and lead to changes in pH unrelated to the movement of hydrogen or hydroxyl ions. Transport of bicarbonate would not affect the pH since the level of bicarbonate in the medium is kept constant by replacement from the gas stream.

When sodium-free conditions were required, potential difference was measured in Tris-HCl (pH 7·4) buffer (Gomori, 1971) whereas acidification, because of the excessive buffering capcity of Tris, was measured in 300 mM mannitol solutions. Both buffers contained 10 mM glucose and were gassed with the previous mixture. This gassing gave the mannitol solution an acid pH which was adjusted with KOH to pH 7·4, giving this medium a slightly higher potassium content of 8·6 mM.

Physiological methods

A 10 cm section of sac 1 (Barry, Matthews & Smyth, 1961), removed from un-fasted female rats (150–200 g) killed by cervical dislocation, was flushed with 0·154 M saline at room temperature and everted (Wiseman, 1961) over a 1·5 mm diameter plastic rod. Either of two types of *in vitro* preparation was made, involving a cannulated sac (Barry, Smyth & Wright, 1965) or where isotopes were used, the everted sac.

The cannulated sac preparation

This preparation allowed simultaneous measurement of potential difference, intermittent short-circuit current and the mucosal acidification by the jejunum in an open system. The only modifications to the apparatus used by Barry *et al.* (1965) were a lead-off from the main chamber via a micropump to a subsidiary chamber housing a pH electrode and a return of the mucosal fluid from the pH measuring chamber into the main chamber near to the source of oxygenation. Circulation time round the system containing the pH electrode was about 1 min and the rate of perfusion was about 2 ml./min. The whole apparatus was maintained at 37° C.

A 5 cm long section of everted proximal jejunum was tied off and the other end tied on to a glass cannula unit, which allowed for the passage of a chlorided silver wire and an agar bridge into the serosal volume. The serosal compartment was filled with a known volume of fluid measured by weighing the difference in weight of a plastic syringe used to inject the serosal volume. The attached sac was immersed in the incubation chamber containing a known (approximately 20 ml.) amount of buffer.

Potential difference and short-circuit current. These were measured in the usual manner (Barry *et al.* 1965). Balanced salt bridges (3 % agar in 3 M-KCl) were connected via calomel half-cells to a Knick (NG20) voltmeter and the potential difference measured 15 min after the start of incubation. External current was passed

through 1 mm diameter chlorided silver wire at 20 min intervals giving inter-mittent short-circuit current readings.

pH measurements. The pH changes in the mucosal solution were recorded by a Pye-Ingold 405 glass pH electrode in conjunction with a Knick (Lador 27) pH meter. When a constant medium pH was observed, the cannulated sac was introduced into the chamber. The pH was continuously monitored via a multichannel pen recorder (Rikadenki) as was the potential difference and the intermittent short-circuit current. After 2 hr, the everted sac was removed and the mucosal solution back-titrated with 10 μl. aliquots of 1 N-NaOH. Therefore with each sac, the pH changes could be correlated with known amounts of hydrogen ion increase for this specific system, rather than calculated or worked out from a nomogram as had previously occurred (Blair *et al.* 1975). Where this was not practicable owing to very small changes, as was the case in sodium-free buffers, a nomogram was used for converting pH differences to actual changes in hydrogen ion concentration.

Measurement of solute transfers. During the experiments, 10 μl. aliquots of the mucosal and serosal fluids were taken for subsequent analysis of the sodium and potassium ion content. This was done after 1:2000 dilution in lithium chloride, by flame photometry with an EEL-butane flame photometer.

The everted sac (isotope studies)

Sacs 5 cm long were injected with 0·4 ml. serosally, stored briefly (less than 1 min) in saline at 37° C and allocated to a control or treatment flask at random. All incuba-tions were at 37° C in buffers incubated with 95:5 (v/v) $O_2:CO_2$ gas mixture. After the appropriate incubation time, the sacs were removed and serially washed in three changes of 50 ml. 154 mM saline solution for 2 min in each wash. The serosal contents of the sac were drained, weighed and taken for counting. Simultaneously, fluid transport measurements were taken (Barry *et al.* 1961) enabling solute and fluid transfer and final concentration ratio to be calculated. In some experiments, two control sacs were used, one incubated at 37° C, one at 0° C: this was to enable estimation of the amount of solute moving across the intestine solely by diffusion, since label was introduced exclusively to the mucosal surface. Active solute flux was worked out as that over and above solute flux occurring in the control sac incubated at 0° C.

[³H]*glucose experiments.* Tritiated glucose was used in an experiment to determine the effect of 10 mM mucosal aminophylline on glucose transfer. Sacs were incubated in modified Krebs buffer containing 10 mM tritiated glucose (specific activity 7·18 cμ/mM) for 1 hr.

²²*Na experiments.* One experiment was done in which the effect of 10 mM mucosal aminophylline and 10 mM serosal ouabain on the amount of sodium appearing serosally, was investigated. Sacs were incubated in modified Krebs buffer containing 30 mM glucose and isotopic sodium (specific activity 0·06 μc/mM) for 15 min. Controls were run at 37° C and at 0° C to estimate the serosal appearance of sodium ion due to diffusion.

Another type of experiment was performed using the ²²Na-containing buffer, in which sacs were incubated in this medium to allow for labelling of the intracellular sodium of the tissues. After 30 min incubation, the sacs were washed by the usual method to remove extraneous label and then transferred to 10 ml. 'cold' Tris buffer. At 3, 6, 12, 24 and 48 min, 50 μl. aliquots of the Tris buffer were taken to monitor the efflux of ²²Na from the tissues.

Counting of samples

In all experiments, the entire serosal contents were mixed with the same volume of 0·6 N perchloric acid, the mixture centrifuged for 15 min at 10,000 rev/min and

a 50 μl. aliquot taken from the supernatant. This was dissolved in 10 ml. liquid scintillant (5·5 g mixture of 2,5-diphenyloxazole, PPO (91 %), and bis-MSB (9 %), dissolved in 1 l. toluol containing 15 ml. Brosolv (Beckman) as a solubilizer and counted on a Beckman (LS 150) scintillation counter.

Expression of results

Tissues were dried to a constant weight in a drying oven at 100° C. All ion movements and electrical data, originally expressed per milligram tissue dry weight, were converted to per square centimetre by nomogram (Lucas & Johnson, 1974). All results are given as the mean, plus one s.E. of the mean, with the number of animals in parentheses.

Viability of the everted preparations

The viability was checked by measuring transmural potential difference and glucose transport. A sustained potential difference averaged 5·4 ± 0·7 (8) mV over the second hr during incubation in the modified Krebs buffer containing 30 mM glucose. In the same buffer containing 10 mM glucose, mucosal glucose transfer was 59 ± 11 (5), μg/mg tissue dry weight causing a final glucose concentration ratio of 3·28 ± 0·30 (5). These values are usual for proximal jejunum (Barry *et al.* 1961; Barry, Dikstein, Matthews, Smyth & Wright, 1964) and indicate adequate physiological function.

Statistical procedures

Except where not feasible (cannulated sac experiments), significance was calculated by Student's paired *t* test, otherwise by unpaired *t* tests (Fisher, 1971). Regression details and the significance of slopes and intercepts were calculated according to Mather (1964).

RESULTS

Transmural potential difference, short-circuit current and mucosal acidification were measured in the proximal rat jejunum to see whether any correlation could be found between acidification and the electrogenic events that occur in proximal jejunum. The buffer contained 30 mM glucose which is the optimal concentration for both transmural potential difference (Barry *et al.* 1964) and maximal rates of acidification (Blair *et al.* 1975). The potential difference which rose in the control sacs to sustained values characteristic of proximal jejunum (Barry *et al.* 1961) was reduced within min (Fig. 1 and Table 1) by 10 mM mucosal aminophylline or 10 mM serosal ouabain to values which persisted throughout the second hour. The action of these inhibitors was associated with a significant increase in tissue resistance (Fig. 1 and Table 1). There was also a marked decrease in the short-circuit current on exposure to either inhibitor (Fig. 2 and Table 1), where the initially higher short-circuit current which fell after 20 min in the controls to a stable value, could be significantly reduced to very low levels. This inhibition is even more pronounced when the mean short-circuit current for the second hour is considered.

·Mirroring the initial slight fall in short-circuit current, the rate of acidification also quickly fell to a constant stable value. Both mucosal
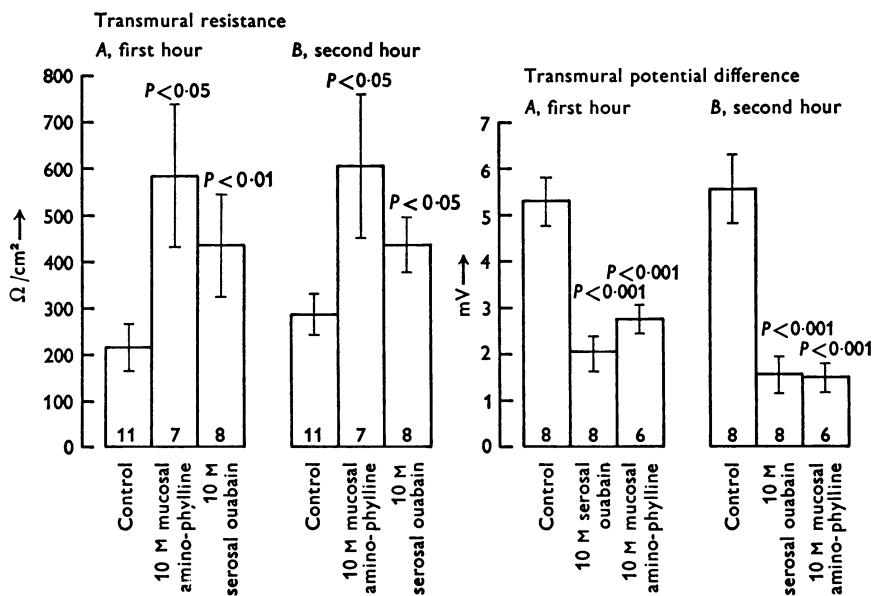
Fig. 1. Effect of serosal ouabain and mucosal aminophylline on transmural resistance and potential in the rat proximal jejunum. Results expressed as mean ± S.E. of mean; number of animals given at foot of each column. Incubated in modified Krebs–Henseleit with 30 mM glucose.

TABLE 1. Collected data from Figs. 1 and 2 showing the effect of 10 mM mucosal aminophylline and 10 mM serosal ouabain on the transmural potential difference, resistance, short-circuit current and acidification (averaged over 1 hr) in rat proximal jejunum incubated at 37° C in modified Krebs–bicarbonate buffer containing 30 mM glucose

	Short-circuit current (μA/cm^2)	Resistance (Ω/cm^2)	Potential difference (mV)	Acidification (μg/mg/per hour)
First control				
Control	$36\cdot1 \pm 8\cdot4$ (11)	238 ± 50 (10)	$5\cdot3 \pm 0\cdot5$ (8)	$0\cdot37 \pm 0\cdot08$ (8)
Ouabain	$8\cdot4 \pm 2\cdot3$ (8)	571 ± 87 (8)	$2\cdot0 \pm 0\cdot3$ (8)	$0\cdot13 \pm 0\cdot05$ (8)
Significance	$P < 0\cdot02$	$P < 0\cdot01$	$P < 0\cdot001$	$P < 0\cdot01$
Aminophylline	$11\cdot2 \pm 4\cdot6$ (7)	584 ± 154 (7)	$2\cdot7 \pm 0\cdot3$ (6)	$0\cdot10 \pm 0\cdot05$ (7)
Significance	$P < 0\cdot05$	$P < 0\cdot05$	$P < 0\cdot001$	$P < 0\cdot02$
Second hour				
Control	$21\cdot6 \pm 4\cdot4$ (11)	280 ± 48 (10)	$5\cdot4 \pm 0\cdot7$ (8)	$0\cdot23 \pm 0\cdot04$ (8)
Ouabain	$3\cdot7 \pm 0\cdot8$ (8)	521 ± 53 (8)	$1\cdot6 \pm 0\cdot4$ (8)	$0\cdot08 \pm 0\cdot02$ (8)
Significance	$P < 0\cdot01$	$P < 0\cdot05$	$P < 0\cdot001$	$P < 0\cdot01$
Aminophylline	$3\cdot8 \pm 1\cdot3$ (7)	639 ± 162 (7)	$1\cdot5 \pm 0\cdot3$ (6)	$0\cdot04 \pm 0\cdot01$ (7)
Significance	$P < 0\cdot01$	$P < 0\cdot05$	$P < 0\cdot001$	$P < 0\cdot005$

aminophylline, as had been previously reported (Blair *et al.* 1965), and serosal ouabain caused permanent reductions in the rate of acidification although some evidence of recovery was seen with ouabain where the rate of acidification after 100 min was significantly higher ($P < 0.02$) than that at 80 min. Since the fall in acidification paralleled the reduction in short-circuit current, apart from this later period of apparent recovery under ouabain inhibition not seen with the short-circuit current, the acidification
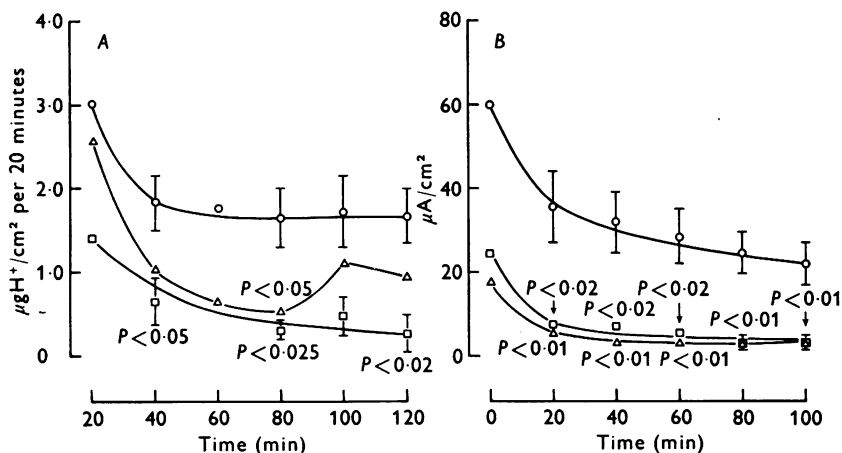
Fig. 2. Effect of serosal ouabain and mucosal aminophylline on: *A*, hydrogen ion production; and *B*, short-circuit current in the rat proximal jejunum. In both: $\bigcirc$, control, $n = 10$; $\triangle$, ouabain, $n = 8$; and $\square$, aminophylline, $n = 7$. Results given as mean $\pm$ s.e. of mean. Incubated in modified Krebs–Henseleit with 30 mM glucose.

over each 20 min period was plotted against short-circuit current measurements made 5 min before the end of this period. A high degree of correlation ($P < 0.01$) was found (Fig. 3) between both variables ($r = 0.8596$), with no significant intercept. On converting short-circuit current to μequiv/cm^2 per hour, the ratio of equivalents of hydrogen ion produced to equivalents of netshort-circuit current was 3·37. This means that although acidification and net short-circuit current seem directionally and stoicheiometrically not related, a definite correlation does exist between the two phenomena. This correlation was confirmed by taking all values at 60 min when it might be argued that the effect of the initial high values of short-circuit current would not disproportionately affect the regression. In this case, there was a high degree of correlation ($r = 0.4912$, $n = 25$, $P < 0.01$) giving a ratio of acidification to short-circuit current of 4·0.

Sodium and potassium ion transfer was also measured during this experiment to see if the movement of these ions correlated with either

short-circuit current or acidification and their alterations by inhibitors. No correlation was noted with either short-circuit current or acidification and net sodium movement, measured photometrically. There was a significant uptake of sodium mucosally and a very small uptake from the serosal compartment (Table 2) by the tissues as has previously been demonstrated under open-circuit conditions (Barry *et al.* 1965): both net uptakes were unaffected by either ouabain or aminophylline. The action of both the inhibitors was tested on the unidirectional active mucosal to serosal
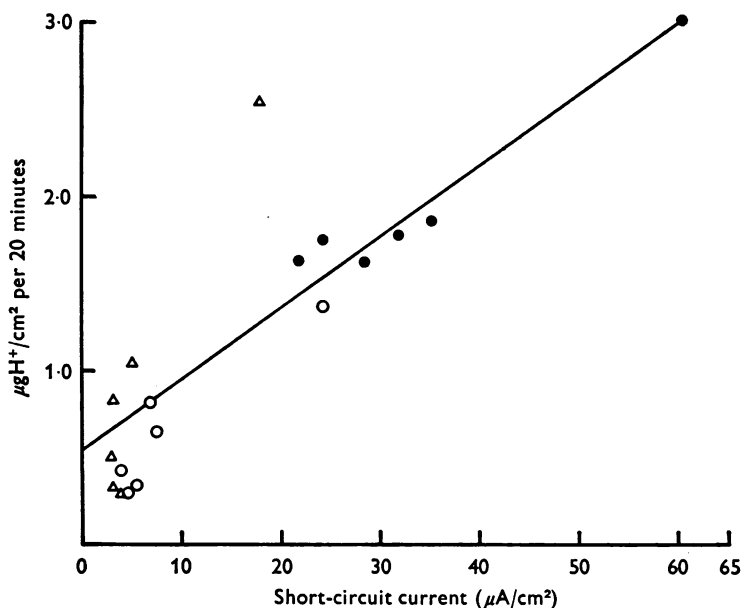
Fig. 3. The regression of acidification on short-circuit current. Here: control, ●; ouabain, △, aminophylline, ○; and $n = 18$, $a = 0.574$, $b = 0.0414$, $r = 0.8596$ and $P < 0.001$. Data from Fig. 2.

sodium flux (J_{ms} Na) which was about three times the value for acidification. It was found however that (Fig. 4) whereas 10 mM serosal ouabain reduced the appearance of sodium in the serosal volume (J_{ms} Na) to half of the control value, 10 mM mucosal aminophylline was completely without effect. The significance of this result is that aminophylline can reduce short-circuit current and acidification without perceptibly altering the unidirectional sodium flux assumed to be one of the main components of the short-circuit current. An exchange therefore between serosally directed sodium ion movement and acidification is very unlikely.

Potassium ion movements (Table 3) showed more correlation with acidification than the previous sodium ion movements. In the control situation,

TABLE 2. Sodium movement in the rat proximal jejunum (experimental conditions as in Table 1)

	Mucosal uptake (after 1st hour)		Mucosal uptake (after 2nd hour)		Serosal transfer (after 2nd hour)	
	μequiv/cm²	μequiv/mg	μequiv/cm²	μequiv/mg	μequiv/cm²	μequiv/mg
Control	185 ± 23 (10)	6·12 ± 0·76 (10)	278 ± 65 (9)	9·50 ± 2·31 (9)	−3·1 ± 1·3 (9)	−0·10 ± 0·08 (9)
10 mM serosal ouabain	234 ± 74 (8)	8·44 ± 2·85 (8)	422 ± 111 (8)	14·38 ± 4·03 (8)	−3·2 ± 1·0 (6)	−0·10 ± 0·03 (7)
10 mM mucosal aminophylline	214 ± 31 (7)	6·93 ± 1·01 (7)	388 ± 40 (7)	12·45 ± 1·32 (7)	−5·24 ± 0·57 (7)	−0·17 ± 0·02 (7)

TABLE 3. Potassium movement in the rat proximal jejunum (experimental conditions as in Table 1)

	Mucosal uptake (after 1st hour)		Mucosal uptake (after 2nd hour)		Serosal transfer (after 2nd hour)	
	μequiv/cm²	μequiv/mg	μequiv/cm²	μequiv/mg	μequiv/cm²	μequiv/mg
Control	6·37 ± 1·00 (10)	0·24 ± 0·05 (10)	12·34 ± 2·64 (9)	0·47 ± 0·12 (9)	0·69 ± 0·36 (10)	13 ± 5 (10)
10 mM serosal ouabain	3·00 ± 2·68 (8)	0·04 ± 0·05 (8)	12·9 ± 8·34 (8)	0·40 ± 0·26 (8)	0·71 ± 0·19 (6)	22 ± 6 (6)
Significance (compared with control)	n.s.	$P < 0.05$	n.s.	n.s.	n.s.	n.s.
10 mM mucosal aminophylline	−1·30 ± 2·22 (7)	−0·04 ± 0·07 (7)	−1·20 ± 2·82 (7)	−0·04 ± 0·99 (7)	−0·13 ± 0·14 (7)	−6 ± 4 (7)
Significance (compared with control)	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.02$
Significance compared with ouabain)	n.s.	n.s.	n.s.	n.s.	$P < 0.01$	$P < 0.01$

there was a net uptake of potassium ion from the mucosal lumen into the tissue; this uptake was maintained in the second hour. As with the sodium ion, there was an uptake of potassium from the serosal compartment only marginally significantly different ($P < 0.05$) from zero. The amount of potassium ion taken up mucosally is of the same order of magnitude as the amount of hydrogen ion produced and an exchange mechanism might be postulated. Both ouabain and aminophylline paralleling their effect on acidification reduced the potassium uptake to zero during the first hour. Aminophylline inhibition persisted for the second hour whereas the inhibitory effect of ouabain was not seen during the second hour again indicating the reversibility of ouabain inhibition. Only aminophylline had an effect on the small marginal serosal potassium ion transfer, causing a reversal of the direction of ion movement; in contrast, ouabain had a discrepant effect, causing an increase in serosal potassium ion which just failed to be significant. There was some degree of correlation between serosal potassium movement and acidification only where ouabain was present when a significant ($P < 0.05$) correlation ($r = 0.75$) was observed. Mucosal potassium ion movement similarly, correlated ($r = 0.801$) moderately ($P < 0.05$) with acidification when compared over all the three experimental conditions, indicating the possibility of potassium–hydrogen ion exchange.

Since ouabain and aminophylline altered ion movements, the question arose whether their action was primarily on the mechanism causing ion movements or secondarily by restricting glucose entry and hence the amount of metabolizable substrate that presumably provides energy for the ion flows. The effect of 10 mM mucosal aminophylline only on glucose and water transfer was investigated since ouabain can reduce glucose transport and metabolism (Newey, Sanford & Smyth, 1968). As with ouabain, aminophylline (Table 4) reduced gut fluid uptake and mucosal water transfer significantly and reversed the direction of serosal water transfer. Mucosal and serosal glucose transfer (Table 4) was approximately halved and the glucose concentration ratio reduced by 30 %. These reductions in glucose transfer are not as severe as those seen with equimolar amounts of ouabain given serosally (Newey *et al.* 1968) and since glucose was still transported serosally sufficient glucose was available for metabolism.

Previous experiments had demonstrated a significant correlation between the magnitude of the net short-circuit current (but not the direction) and acidification. Experiments were therefore carried out in the absence of sodium ion to see the effects of both inhibitors of acidification on the reversed transmural potential differences obtained in sodium-free media thought to be caused by hydrogen ion secretion (Faelli & Garotta, 1971*a*, *b*).

In the complete absence of the sodium ion contribution (Fig. 5A), a reversed potential difference occurred which was not sustained but fell after an initial peak to zero. Serosal ouabain and mucosal aminophylline appeared to have precisely opposite effects in this situation where ouabain caused an increase and aminophylline a decrease in the transient reversed

TABLE 4. The effect of 10 mM mucosal aminophylline on glucose and water transport in the rat proximal jejunum incubated at 37° C for 1 hr in bicarbonate buffer. Results are expressed as mean ± S.E. of mean (no. of animals)

A, water transport (mg/mg dry wt. per hour)

	Mucosal water transfer	Gut fluid uptake	Serosal water transfer
Control	4·88 ± 0·45 (10)	3·03 ± 0·20 (10)	1·86 ± 0·32 (10)
Aminophylline	1·24 ± 0·20 (10)	1·76 ± 0·17 (10)	− 0·64 ± 0·20 (10)
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

B, glucose transport (μg/mg dry wt. per hour)

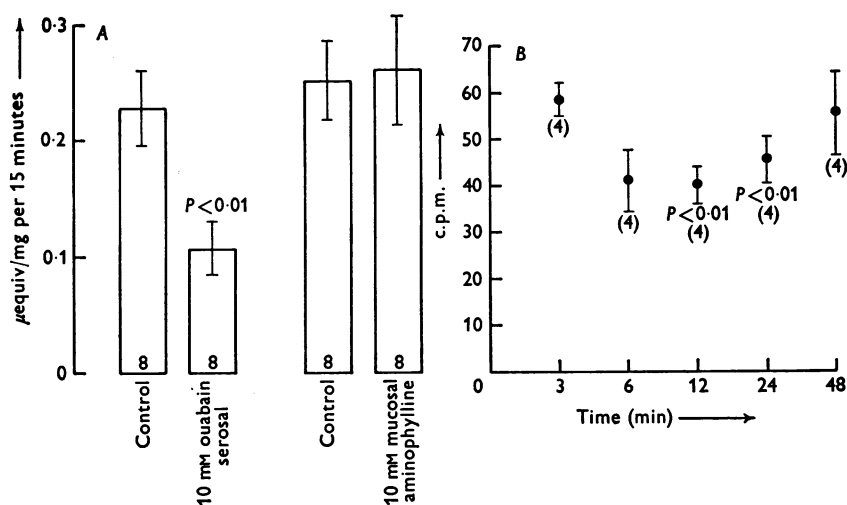|	Mucosal glucose transfer	Serosal glucose transfer	Glucose concentration ratio
Control	59·4 ± 11·0 (5)	19·2 ± 3·4 (5)	3·28 ± 0·30 (5)
Aminophylline	32·4 ± 7·4 (5)	10·0 ± 3·1 (5)	2·27 ± 0·33 (5)
Significance	$P < 0.01$	$P < 0.05$	$P < 0.01$

Fig. 4. Isotopic sodium experiments: A, the effect of 10 mM mucosal aminophylline and 10 mM serosal ouabain on active serosal sodium transfer in sacs of proximal jejunum incubated in bicarbonate buffer containing 30 mM glucose; and B, the efflux of ^{22}Na from sacs incubated in sodium-free Tris buffer containing 30 mM glucose. After 30 min pre-incubation in the previous bicarbonate buffer.

potential differences. The transience of these reversed potential differences in buffer totally devoid of sodium might be due to gradually lessening diffusion of sodium from out of cells, generating a diffusion potential. To investigate this point, the diffusion characteristics of cellular sodium into sodium-free buffer were measured by observing the efflux of isotopic sodium from everted sacs, over the same time period.
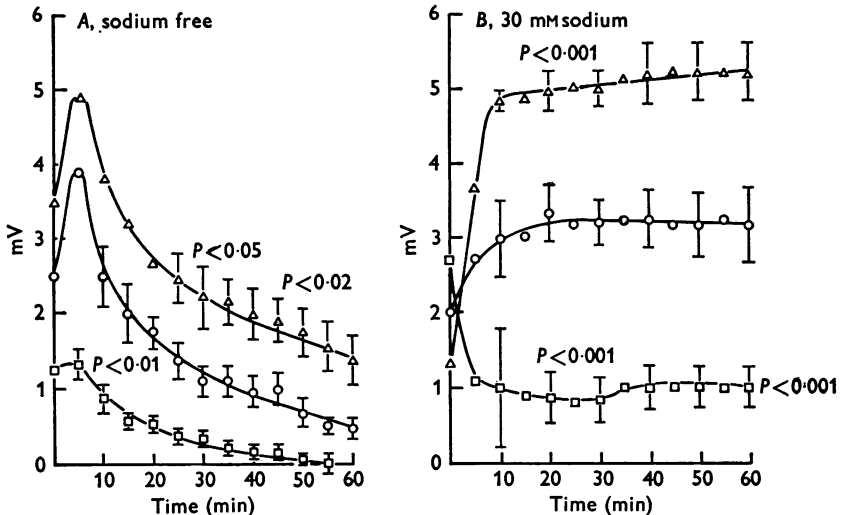

Fig. 5. The action of serosal ouabain and mucosal aminophylline on reversed transmural potentials in proximal jejunum incubated in (A) sodium free and (B) 30 mM sodium-Tris buffer. In both: control, $\bigcirc$, $n = 5$; ouabain, $\triangle$, $n = 5$; and aminophylline, $\square$ and in (A) $n = 5$ and (B) $n = 4$. Results expressed as means $\pm$ S.E. of mean.

Sacs were incubated in Na^{22}-containing modified Krebs–bicarbonate buffer to label the intracellular sodium pool. The sacs were given a thorough washing in saline to remove extraneous label and then incubated in Tris buffer (as used for the reversed potential difference experiments) to study the efflux of isotopic sodium. Rapid diffusion of intracellular sodium into the Tris buffer occurred within the first 3 min of incubation (Fig. 4B) which might be expected to cause the peak of the transient reversed potential differences, if these were generated by the sodium efflux. After this initial rapid efflux, there was again an uptake of sodium from the buffer which becomes significant at 12 min. This initial re-uptake, calculated to be about 8 μequiv sodium/mg dry wt per hour, which corresponds well with the usual figure under normal conditions (Table 2), fell over the 6–12 min period, to 0·2 μequiv. After this time the efflux of sodium re-asserted itself: this has no parallel in the transient reversed

potential difference curves (Fig. 5*A*) where if sodium efflux were to be their cause, a second peak in the reversed potential difference might reasonably be expected. More likely is that the fall away in the reversed potential differences is due to the gradual cessation of normal metabolic activity which occurs in sodium-free buffers (Jordana & Igea, 1970; Ponz & Lluch, 1971) as evidenced by this decline in sodium pumping activity. For this reason the experiments were repeated in Tris containing 30 mM sodium (the approximate intracellular concentration) to prevent cellular sodium loss. In this buffer the potential differences remained stable for at least 1 hr (Fig. 5*B*) and as before, 10 mM serosal ouabain increased and 10 mM mucosal aminophylline decreased the stable reversed. Finally, to see whether acidification persisted in the absence of buffer sodium ion, it was measured in sodium-free mannitol solution. It was not possible to measure acidification in the previously used Tris buffer because of the excessive buffering capacity of this buffer. Acidification persisted in this buffer, occurring at a rate of 0·18 μg/mg per hour, about half the usual value in normal Krebs–bicarbonate and indicated that acidification may be an electrogenic event causing a potential difference in the reversed direction to the usual direction of the short-circuit current which only becomes apparent in the absence of sodium ion.

DISCUSSION

Much attention has focused on the relation of sodium ion to short-circuit current in rat jejunum whereas the contribution of other ions, particularly hydrogen ion, has remained unconsidered. The present experiments demonstrated a correlation between acidification and short-circuit current inferring that hydrogen ion production is somehow linked to an electrogenic portion of the total short-circuit current. Previous experiments have ruled out the hexose entry mechanism as the cause of acidification (Blair *et al.* 1975), yet restricted glucose availability due to impaired glucose entry could cause reduced acidification. Aminophylline reduced serosal and mucosal glucose transfer by half; yet glucose was still extruded serosally, indicating that the metabolic requirements of the cells were being met. It is unlikely therefore that the curtailment in acidification is due to the unavailability of glucose substrate. The diverse effects on ion movements of both inhibitors seems further to exclude a general restrictive effect on intermediate metabolism and indicate that aminophylline and ouabain intervene directly in the ion flows across the small intestine. Consequently, their action on acidification, short-circuit current and ion movements provides evidence for certain ion exchange mechanisms and precludes others.

Sodium–hydrogen ion exchange

Sodium–hydrogen ion exchange has been proposed as a mechanism for acidification in human and rat jejunum (Wilson & Kazyak, 1957; Turnberg et al. 1970; Klotz & Schloerb, 1971). The net serosally directed sodium flux, thought to be a component of the short-circuit current in the jejunum (Taylor, Wright, Schultz & Curran, 1968; Munck, 1972), consists of a unidirectional serosally-directed flux attenuated by shunt pathways (Munck & Schultz, 1974). Estimates for the unidirectional (J_{ms}) sodium flux agree with the previous estimates (Taylor et al. 1968; Munck, 1972) and the degree of inhibition by ouabain of $0{\cdot}4\,\mu$equiv/mg per hour agrees with the corresponding inhibition of $0{\cdot}3\,\mu$equiv/mg per hour for acidification inviting speculation as to partial coupling of acidification with about 25 % of the total J_{ms} sodium flux. Although these figures agree well with the concept of sodium–hydrogen exchange, often postulated (Turnberg et al. 1970) aminophylline, reduced acidification without any perceptible effect on the J_{ms} sodium flux. Since aminophylline can dramatically reduce the short-circuit current and acidification with no effect at all on sodium flux (which may also indicate that the sodium ion contributes little to the short-circuit current in the proximal jejunum), a compulsory exchange of sodium with hydrogen ions is unlikely.

Potassium–hydrogen ion exchange

There was significant mucosal uptake and serosal secretion of potassium ion against the prevailing electrical and also presumably the chemical gradient since estimates for intracellular potassium concentration are of the order of 40 mM and above (Koopman & Schultz, 1969; Remke, Luppa & Muller, 1972). The mucosal potassium uptake could account for two thirds of acidification if it were involved in some form of coupling. Both inhibitors affected this uptake, unlike sodium movement. Also the resumption of mucosal potassium uptake in the second hour after ouabain inhibition may be related to the recovery of acidification also seen during the same period.

Serosal potassium movement may also function in mucosal acidification. In the absence of Na–K-pump mediated sodium-coupled potassium ion movements, serosal transfer of potassium ion correlated (not stoicheiometrically) with mucosal hydrogen ion production. Aminophylline caused a reversal of the direction of potassium ion transfer causing potassium ion to be taken up from the serosal solution. This may indicate the existence serosally of two pumps working in parallel involving potassium ion, an aminophylline-sensitive K^+/H^+ exchange ejecting potassium ions serosally and taking up hydrogen ions, and the conventional ouabain–

sensitive Na^+/K^+ pump, ejecting sodium serosally and taking up potassium ions (see Fig. 6). This serosal K–H exchange pump could be a way of reducing the intracellular pH to usual values enabling further mucosal acidification. On balance there seems therefore to be more evidence for a jejunal potassium–hydrogen ion exchange than for a sodium–hydrogen ion exchange.
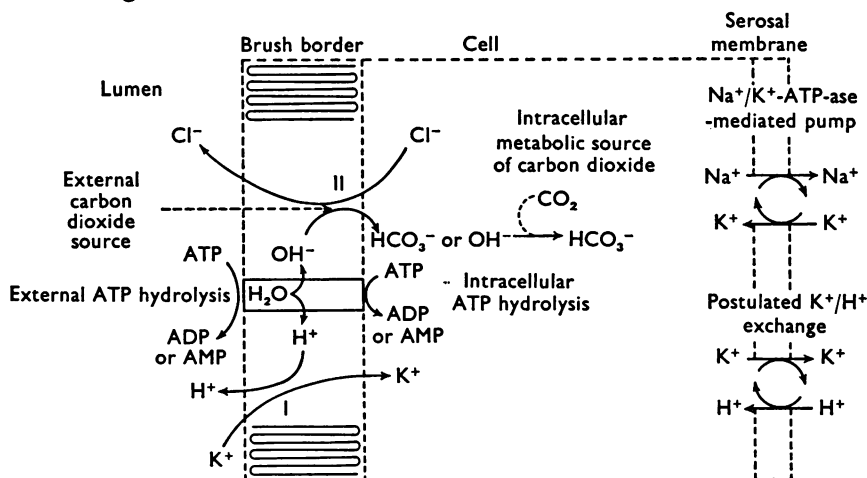

Fig. 6. A diagram of the mechanism of acidification in proximal rat jejunum showing two possible electrogenic steps (I and II) and a K^+–H^+ exchange mucosally and serosally.

Acidification and the reversed transmural potential difference

The reversed potential difference that occurs in sodium-depleted media has been ascribed to diffusion potentials (Barry & Eggenton, 1972). When low-sodium buffers were used, these reversed potential differences were sustained at constant levels for at least 1 hr; it seems unlikely that back diffusion of ions from out of the tissues would continue for this length of time. Also when 30 mM sodium–Tris buffer was used, a reduction in the potential difference might be expected and not the constant values, since the concentration gradient down which the sodium ion has to diffuse was less in this case. The sustained reversed potential difference seen in low-sodium buffer could be due to acidification since acidification does occur in sodium-free buffer. However, the cause of the potential difference is more likely to be anions moving serosally. If the reversed potential differences reflect accurately ion flows, then ouabain which reduced acidification in a sodium-free buffer, ought to have reduced the reversed potential difference (which was not the case).

Preliminary experiments indicate that in the complete absence of sodium,

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10 mM serosal ouabain will increase the transfer of bicarbonate across into the serosal compartment (M. L. Lucas, unpublished). The transfer, consequent on acidification, of bicarbonate anions after charge separation being the cause of the reversed potential difference would explain the increase by ouabain of the reversed potential difference. If the reversed potential differences were caused by hydroxyl ion movement into the cells buffered by either an intra- or extracellular carbon dioxide source, it would also explain why these potential differences decline in buffers free of bicarbonate (Faelli & Garotta, 1971b).

A model for jejunal acidification

A model has been proposed for acidification (Blair et al. 1975) involving charge separation (see Fig. 6) of the hydrogen ion from its accompanying anion at the luminal membrane, caused by ATP hydrolysis. H^+/K^+ exchange would explain the effects of various hexoses on the intracellular potassium content, where unmetabolizable sugars, which do not cause acidification, e.g. galactose, cause loss of cell potassium whereas metabolizable sugars by H^+/K^+ exchange restore or cause elevations in the intracellular potassium level (Koopman & Schultz, 1969; Remke et al. 1972).

Although conjectural, it is more likely that the underlying mechanism to acidification that is the electrogenic step is bicarbonate ion uptake (and not hydrogen ion extrusion) which could be balanced by movement of chloride ion (Taylor et al. 1968; Munck, 1972) as becomes evident at low rates of sodium transport (Powell et al. 1971).

The transfer of many weakly ionizing substances, e.g. benzoic acid and short-chain fatty acids (Jackson et al. 1974; Bloch, Haberich & Lorenz-Meyer, 1972) is often not able to be explained solely in terms of passive processes. Models have been proposed (Blair et al. 1974) involving secretion of hydrogen ions which allow anionic forms of these substances to cross the gut as the neutral species in accordance with the principles of nonionic diffusion. It may be that the alternative mechanism, i.e. that of hydroxyl ion uptake (underlying acidification) with subsequent exchange of this ion with the ion to be transported may have to be considered as the system which accelerates the transfer of weak electrolytes.

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